

Supplemental Data

The general health and behaviors of Sp4 hypomorphic mice has been described in our previous publication (3). The general health including some sensory and neurological functions was tested again in the Sp4 hypomorphic mouse cohort used in the Barnes maze test. There was no genotypic difference in any measure between wildtype and Sp4 hypomorphic mice.

The first few measures were taken by observing each mouse in its home cage prior to any handling. These observations included an examination of coat condition, a check for barbered hair, piloerection, body tone, skin color, and limb tone. The home cage behavior prior to cage opening was also noted (i.e. solitary sleeping) and any evidence of fighting or aggression was noted. Each mouse was petted while still in the cage and it was noted if the mouse moved away. Then it was lifted by its tail and its behavior examined for passivity, trunk curl, and forepaw reaching. The mouse was scruffed for a firm hold and struggling and vocalization were noted. While scruffed, eye blink, ear twitch, whisker and toe pinch reflexes were assessed. The muzzle was examined for missing whiskers. A dowel was placed in front of the muzzle and biting behavior was scored. Finally, the mouse was placed in an empty, clean cage and freezing on transfer, wild running and stereotypies were noted if any. Cage exploration was scored.

Supplemental Figure 1. RT-PCR analysis of alternative splicing in NMDAR1 mRNA.

(left figure)

lane 1, 1 kb plus marker; lane 2 and 10, (P1-f, E2r); lane 3 and 11, (P2-f, E2r); lane 4 and 12, (P3-f, E2r); lane 5 and 13, (P4-f, E2r); lane 6 and 14, (P5-f, E2r); lane 7 and 15, (E4f, E6r); lane 8 and 16, (E20f, E22r1); lane 9 and 17, (E20f, E22r2)

NR1-E1-P1-f:	5' CACACTGGTG AACAGCCAAA GAG	3'
NR1-E1-P2-f:	5' CC TCGAACTCAG AAATCTGCCT G	3'
NR1-E1-P3-f:	5' CCAGCGGTAT AGAGGGACAG G	3'
NR1-E1-P4-f:	5' TATG CTAACGCGCG TGCACATG	3'
NR1-E1-P5-f:	5' ATCGGGACCA GTCGCACAGT CCA	3'
NR1-E2-r	5' GACTAACTAG GATAGCGTAG AC	3'
NR1-E4-f:	5' AGATGATGCG CGTCTACAAC TGG	3'
NR1-E6-r:	5' GCCGTCACAT TCTTGGTTCC TGG	3'
NR1-E20-f:	5' A CGTGTGGAGG AAGAACCTGC AG	3'
NR1-E22-r1:	5' CAATAGCGCG TCGCGGCAGC ACTGTG	3'

NR1-E22-r2: 5' TGGGCCAG GCCGCTGTCC CGTG 3'

(right figure)

lane 1, 1 kb plus marker; lane 2 and 9, (E2f, E5r); lane 3 and 10, (E5f, E7r); lane 4 and 11, (E7f, E10r); lane 5 and 12, (E10f, E13r); lane 6 and 13, (E13f, E16r); lane 7 and 14, (E16f, E19r); lane 8 and 15, (E19f, E22r2)

NR1-E2-f: 5' CTACCCGAATGTCCATCTACTCTGA 3'

NR1-E5-f: 5' GATCTCTGGGAATGCCCTGCGCTA 3'

NR1-E5-r: 5' AGAGCCAGTC ATGTTTCAGCA TCG 3'

NR1-E7-f: 5' CAAGTGGGCATCTACAATGGTACC 3'

NR1-E7-r: 5' TCTGCATACT TGGAAGACAT CAG 3'

NR1-E10-f: 5' CTTGTGGCAGATGGCAAGTTTGG 3'

NR1-E10-r: 5' AACGCAGAAG CCATAACAGC ACT 3'

NR1-E13-f: 5' TCCTGCTCAACTCTGGCATTGG 3'

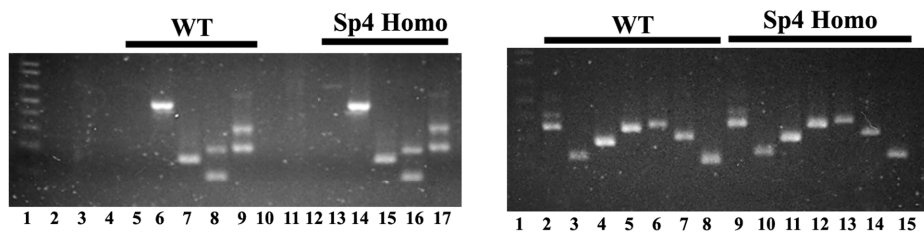
NR1-E13-r: 5' CTCCTCGCTG TTCACCTTAA ATC 3'

NR1-E16-f: 5' AAAATGTGTCCCTGTCCATACTC 3'

NR1-E16-r: 5' ACAGCTGAGT CCCAGATGAA GGC 3'

NR1-E19-f: 5' GCTTCAAGAGACGTAGGTCCTCC 3'

NR1-E19-r: 5' GGCTCTGCTC TACCACTCTT TC 3'



Zhou et al. Supplemental Figure 1